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# Characteristics and emulsifying properties of acid and acid-heat induced egg white protein

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ABSTRACT

This study investigated the influence of acid (pH 3.0) or moderate heat (60 °C, 15 min, pH 3.0) treatments on physicochemical characteristics (ζ-potential and hydrophobicity), structure (Raman spectra) and emulsifying capacity of EWP after re-adjusted to various pH. Simultaneously, the stability of the emulsions prepared with acid and acid-heat induced EWP (AEP and AHEP) was tested by measuring particle diameter and microstructure of the droplets after salt (0–200 mM NaCl) and heat (90 °C, 30 min) treatments at pH 3.0–4.2. The results revealed that the emulsifying capacity of EWP was enhanced after acid or acid-heat treatment with more hydrophobic amino acids and charged groups exposed on the surface, but little changes in secondary structure. Proteins adsorbed at the oil-water interface were mainly ovotransferrin and ovalbumin. The emulsions made with AEP and AHEP were stable to droplet aggregation with no phase separation during storage for 3 weeks at pH below isoelectric point (pI ≈ 5.0), but exhibited some aggregation at pH near or above pI. The heat stability of emulsions depended on pH and thermal history. The emulsions made with AHEP performed better heat stability than AEP-stabilized emulsions. The salt stability of emulsions depended on the net charge. At pH 3.0, the emulsions were stable in the presence of ≤100 mM NaCl. At other pH, the emulsions showed good tolerance to 50 mM NaCl. These results have important implications for the formulation and production of emulsion based acid products, using egg white protein as emulsifier.



## 1. Introduction

Egg white contains 9.7–10.6% protein in weight, the main constituents of which, for example, ovalbumin, ovotransferrin, ovomucoid, and lysozyme, are globular nature. A number of intramolecular disulfide bonds as well as hydrophobic interactions between nonpolar amino acid groups buried inside the molecular structure of these globular proteins (Drakos & Kiosseoglou, 2006). Thus egg white proteins (EWP) possess multiple functional properties, such as foaming, emulsification, gelation and binding adhesion, due to amphoteric nature and film-forming abilities (Huntington & Stein, 2001). Benefiting from good gelation and foaming characteristics, egg white proteins are widely used in foods such as bakery products, meringues, meat products and cookies. However, its emulsifying potential is generally considered rather poor compared to other proteins like whey protein and soy protein, which restricts its application in emulsion system.

Physicochemical characteristics like surface hydrophobicity and net charge of proteins play an important role in determining their emulsifying abilities (Qian & McClements, 2011). Surface charge of the protein influences diffusion rates to the interface through affecting the solubility of the proteins within the aqueous phase (Delahaije, Wierenga, van Nieuwenhuijzen, Giuseppin, & Gruppen, 2013). Surface hydrophobicity performs as one of the key factor of high emulsion capacities by influencing the ability for the protein to adsorb to the oil side of the interface (Kim, Decker, & McClements, 2005). Ovalbumin as the main components of egg white protein is constituted of 385 amino acids (molecular weight of 43 kDa), of which a half are hydrophobic and a third are charged, the majority acidic conferring to the protein a pI of 4.5 (Croguennec, Renault, Beaufils, Dubois, & Pezennec, 2007). But in neutral and alkaline

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conditions, most hydrophobic amino acid residues hide inside the molecule structure, thus very little are exposed on the surface. Therefore, EWP performs strong hydrophilic properties and poor emulsifying ability. However, previous study showed that at pH 3.0, emulsifying activity of ovalbumin was relatively high correlate with the greater surface hydrophobicity and flexibility of the molecule (Mine, Noutomi, & Haga, 1991). In extremely acidic conditions, the hydrophobic groups on the surface of ovalbumin may reduce the kinetic barrier for adsorption of molecule to the interface (Alizadeh-Pasdar & Li-Chan, 2000). Several studies revealed that globular protein may undergo partial unfolding and then possess a molten globule conformation after being subjected to an extreme acidic pH condition, and become more flexible but yet retain a relatively intact conformation (Sivakumar & G, 2007). Significant functionality improvements caused by structural changes for soy protein have been reported following acid or alkali treatments (Jiang, Chen, & Xiong, 2009). Previous study also showed that, pH-induced unfolding can significantly modify the rheological properties of foams stabilized by egg ovalbumin, owing to more exposed -SH and hydrophobic chains. However, the impact of extreme acid or alkaline unfolding processes on conformation and emulsifying ability of EWP in oil-water system is not well documented.

In addition, emulsion ability of ovalbumin can be improved significantly correlating to the increase of hydrophobicity after heat-treatment (Kato, Osako, Matsudomi, & Kobayashi, 1983). Heat pre-treatments can cause partial or complete unfolding of the protein's tertiary conformation to expose buried hydrophobic amino groups to the surface, and in the case of globular proteins, open up its conformation to increase its excluded volume and flexibility (Raikos, 2010). These indicate that egg white protein may effectively aid in the formation and physical stabilization of these emulsion systems relatively after extreme pH-shifting or pre-heat treatment. However, thermal stability of egg white protein is very poor, which limits the application of thermal modification technology in improving the functional properties of EWP. In comparison, acid-heat treatment is more moderate, inducing tertiary structure transition with little changes in secondary structure. This method may be more efficient in inducing partial expansion of egg white protein, avoiding aggregates.

This research investigated the effect of acid and acid-heat treatment on the physicochemical characteristics, structure and emulsion properties of egg white protein, in order to further reveal the relationship between structural modification and functional improvement. Additionally, salt and heat stability of the emulsions was also studied to provide theoretical basic for further application of EWP in emulsion food systems.

## 2. Materials and methods

### 2.1. Materials

Hen eggs were supplied by an enterprise called Rongda (Xuancheng, Anhui, China). For the preparation of the emulsion, Arowana sunflower oil was bought from a local supermarket and used without further purification. HCl, NaOH, sodium chloride (NaCl), sodium 8-anilino-1-naphthalenesulfonate (ANS), sodium azide ($NaN_3$) was obtained from Sigma–Aldrich (St. Louis, MO). All reagents were of analytical grade.

### 2.2. Preparation of egg white protein

Egg white separated from washed hen egg was dispersed in equal volume of distilled water and adjusted to pH 5.0 with 0.5 M HCl. After stirring for 0.5 h and regulating pH to 5.0, the suspension was centrifuged at 5500 g for 15 min at room temperature, and then the supernatant was collected as nature EWP sample for future use. Acid induced EWP was prepared as follow: the supernatant protein solution was titrated to pH 3.0 with 2 M HCl, held at this pH for 1 day at 4 °C to induce partial unfolding, and then titrated back to pH 5.0 with 2 M NaOH to allow refolding. Then, the sample was freeze-dried and stored in desiccator for further examination. Acid-heat modified protein was prepared as follows: the titrated EWP (pH 3.0) was heated in a shaking water bath at 60 °C for 15 min, then removed and immediately cooled to 4 °C with ice-water bath. The heated sample was titrated to pH 5.0 and freeze-dried for further usage. The nature egg white protein was referred “NEP”, the acid induced protein was referred “AEP” and the acid-heat synergistically modified protein was referred “AHEP”.

### 2.3. Surface hydrophobicity

Surface hydrophobicity of NEP, AEP and AHEP solutions at different pH (3.0, 3.4, 3.8, 4.2, 4.8, 6.0) was measured according to the procedure of Alizadeh-Pasdar et al. with a slight modification, which uses a 8-anilino-1-naphthalenesulfonate (ANS) probe to interact with hydrophobic moieties on the protein's surface to give a fluorescent signal (Alizadeh-Pasdar & Li-Chan, 2000). Each protein solution was diluted to concentration of 0.08%. Twenty microliters of a 8 mM ANS solution dissolved in phosphate buffer (50 mM, pH 7.0) was added to 4 mL of each protein solution, vortexed for 15 s, and then kept in the dark for 15 min. The solution was excited at 390 nm, and the emission spectrum was measured from 400 to 600 nm using F-7000 spectrofluorimeter (Hitachi, Japan). The emission and excitation slits were set to 5 nm, and the measurements were performed at 25 °C. The maximum area of the fluorescence spectrum was corrected with the area of the buffer. Subsequently, the relative exposed hydrophobicity was expressed as the area of the sample relative to the area of the sample with the maximum area.

### 2.4. Raman spectroscopic measurement

Raman spectra of NEP, AEP and AHEP was measured. The measurements were made on the surface of protein membrane nature dried on foil, using LabRAM HR Evolution (HORIBA Jobin Yvon S.A.S.Company FRA). The slides wrapped in foil were placed under a microscope with a 50× objective to collect the Raman scattering from the samples. The instrument was equipped with a 785 nm laser (Invictus, Kaiser Optical Systems Inc., Michigan, USA). The laser was focused on each sample and the laser power on the sample was 334 mW. The spectra were acquired using an average spectrum of 2 scans each with an exposure time of 100 s and were stored as Raman shifts in the range of 400–1800 $cm^{-1}$. The results were normalized and corrected baseline with Origin. 8.0.

### 2.5. Preparation of emulsion

To compare the emulsion capacity of NEP, AEP, and AHEP, the freeze-dried powder of each sample was dissolved in distilled water at a ratio of 0.3%, 0.6%, 1.0%, 1.5% (m/v) and the pH was adjusted to 3.8 close to acidity of most drinks. Then the mixtures of protein solution and oil at a volume ratio of 9:1, containing 0.02% (w/v) sodium azide as an antimicrobial agent, were pre-homogenized for 2 min at 11,000 rpm using an Ultra-Turrax blender (IKA T25 Basic, Staufen, Germany) equipped with a 12 mm diameter head. After this treatment, coarse emulsions were obtained. The resulting emulsions were homogenized with a high-pressure homogenizer (APV1000, APV Co., Crawley, U.K.) at 50 MPa for 3 times. Samples were sealed and stored at 4 °C until analysis.

To investigate the tolerance of emulsions prepared with